Scientific Opinion on Flavouring Group Evaluation 212 Revision 3 (FGE.212Rev3): ,-unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 212 Revision 3 (FGE.212Rev3): α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

The scientific opinion FGE.212 Revision 3 published on 28 May 2015, replaces FGE.212 Revision 2 published on 19 February 2014[4]

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate the genotoxic potential of 22 flavouring substances from subgroup 2.6 of FGE.19 in the Flavouring Group Evaluation 212. Based on available genotoxicity data and new genotoxicity data submitted by the Industry, the Panel concluded that genotoxic potential could be ruled out for the six carvone derivatives [FL-no: 02.062, 07.146, 07.147, 09.143, 09.215 and 09.870], the 11 isophorone derivatives [FL-no: 02.083, 02.101, 07.035, 07.098, 07.126, 07.129, 07.172, 07.175, 07.196, 07.202 and 07.255] and the five substances [FL-no: 07.033, 07.094, 07.112, 07.140 and 07.219] from subgroup 2.6 in FGE.212, FGE.212Rev1 and FGE.212Rev3, respectively. Two substances previously included in FGE.212Rev2, vetiverol and vetiveryl acetate [FL-no: 02.214 and 09.821], are no longer supported by Industry. Based on the available data, all 22 substances of this FGE are no longer of concern with respect to genotoxicity and can be evaluated through the Procedure.

KEY WORDS

α,β-unsaturated alicyclic ketones, flavouring substances, safety evaluation, FGE.212, FGE.19, subgroup 2.6

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[1] On request from European Commission, Question Nos EFSA-Q-2013-00992, EFSA-Q-2013-00993, EFSA-Q-2013-01009, EFSA-Q-2013-01010, EFSA-Q-2013-01011, adopted on 5 May 2015.

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[3] The Panel wishes to thank the members of the Genotoxicity Working Group on Flavourings: Mona-Lise Binderup, Claudia Bolognesi, Riccardo Crebelli, Rainer Gürtler, Natália Kovalkovičová, Francesca Marcon, Daniel Marzin and Pasquale Mosesso, for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby and EFSA staff: Annamaria Rossi, Maria Carfi and Maria Anastassiadou for the support provided to this scientific opinion.

[4] The present scientific opinion, FGE.212 Revision 3, includes the additional information on vetiverol [FL-no: 02.214] and vetiveryl acetate [FL-no: 09.821] received after the publication of FGE.212 Revision 2.

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SUMMARY

Following a request from the European Commission, the (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate flavouring substances using the Procedure referred to in Commission Regulation (EC) No 1565/2000 (hereafter 'the Procedure').

The present revision of FGE.212, FGE.212 Rev3, deals with the evaluation of additional genotoxicity data submitted by the Flavour Industry on the representative substance 3-methyl-2-cyclopenten-1-one [FL-no: 07.112]. Furthermore, since the last revision of FGE.212 (revision 2), the Flavour Industry has informed EFSA that in the case of two flavouring substances, vetiverol [FL-no: 02.214] and vetiveryl acetate [FL-no: 09.821], information previously submitted to the European Commission, and on which EFSA's evaluation was based, was incorrect regarding the name, structure and composition of these substances. The use of these two substances as chemically defined flavouring substances is no longer supported by the Industry.

Flavouring Group Evaluation 212 (FGE.212) concerns 22 substances. The 22 substances correspond to subgroup 2.6 of FGE.19. Sixteen of these substances are α,β-unsaturated alicyclic ketones [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 07.146, 07.147, 07.172, 07.175, 07.196, 07.202, 07.219 and 07.255] and six are precursors for such ketones [FL-no: 02.062, 02.083, 02.101, 09.143, 09.215 and 09.870].

In the first version of this Opinion, FGE.212, the Panel expressed the following view:

> "*d*-Carvone [FL-no: 07.146] was found to be genotoxic *in vitro*. However, *d*-carvone was not carcinogenic in mice. Therefore, the Panel concluded that this substance together with the structurally related *l*-carvone as well as carveol and the carvyl derivatives [FL-no: 02.062, 07.147, 09.143, 09.215 and 09.870] could be evaluated through the Procedure.
>
> Isophorone [FL-no: 07.126 (3,5,5-trimethylcyclohex-2-en-1-one)] is genotoxic *in vitro* and since there is some evidence of carcinogenicity in male rats and equivocal evidence of carcinogenicity in male mice and since a non-threshold mechanism could not be excluded based on the data currently available, the Panel concluded that additional data are required for isophorone in order to clarify whether genotoxicity occurs *in vivo* and whether there is a threshold for the effects observed in the target organs in the long-term bioassays. Therefore, an *in vivo* Comet assay in F344/N rats covering these target organs was required in addition to an *in vivo* bone marrow assay with oral application.
>
> Due to structural similarities and lack of data, the remaining substances could not be evaluated through the Procedure [FL-no: 02.083, 02.101, 02.214, 07.033, 07.035, 07.094, 07.098, 07.112, 07.129, 07.140, 07.172, 07.175, 07.196, 07.202, 07.255 and 09.821]. Additional data on genotoxicity were requested for representative substances of this subgroup according to the opinion of the Panel on Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19."

Based on three sets of additional data submitted by the Industry in response to requested data in FGE.212, the Panel concluded that:

- the genotoxicity concern for isophorone [FL-no: 07.126] and the substances structurally related to isophorone [FL-no: 02.083, 02.101, 07.035, 07.098, 07.129, 07.172, 07.175, 07.196, 07.202 and 07.255] could be ruled out.
- New data for 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] can rule out genotoxicity of this substance and four other substances [FL-no: 07.033, 07.094, 07.140 and 07.219].

- In the case of the two substances for which new genotoxicity information was submitted and evaluated in FGE.212Rev2, vetiverol and vetiveryl acetate [FL-no: 02.214 and 09.821], the Flavour Industry has subsequently informed EFSA that information previously submitted to European Commission, and on which the EFSA's previous evaluation was based, was incorrect regarding the name, structure and composition of these substances. The use of these two flavouring substances is no longer supported by the Industry. Accordingly, these two substances will not be considered further in this FGE.
- Based on the available data, the substances considered in this FGE are no longer of concern with respect to genotoxicity and can be evaluated through the Procedure.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavouring is regulated under Regulation (EC) No 1334/2008[5] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation an evaluation and approval are required for flavouring substances.

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[6]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) N° 1565/2000[7].

On 25 November 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 212, Revision 1 (FGE.212Rev1): α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19[8].

The Panel concluded that the argumentation of Industry to expand its conclusion for the six-carbon ring members of subgroup 2.6 also to the cyclopentenyl derivatives in this subgroup [FL-no: 07.033, 07.094, 07.112 and 07.140] was considered too limited, given the lack of support from experimental data. Therefore, additional genotoxicity tests are still required for the representative substance [FL-no: 07.112] already chosen by the Panel. Alternatively, a more thorough explanation (physico-chemical parameters; experimental underpinning) of the proposed similar reactivity of six- and five-membered ring substances should be provided by Industry.

The requested data have been submitted by the applicant.

In addition, the flavouring substance [FL-no: 07.219], trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, was put in FGE.212 (former FGE.19, subgroup 2.6b: α,β-unsaturated aldehydes and ketones and precursors) because of its structure relationship with this group. Although the substance as such is not mentioned in the data submitted by the applicant, the submitted data are likely to be relevant for [FL-no: 07.219] as well.

Therefore, this request covers as well the re-evaluation of trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment of these flavouring substances in accordance with Commission Regulation (EC) No 1565/2000[6].

[5] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

[6] EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

[7] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8–16.

[8] EFSA Journal 2011;9(3):1923.

HISTORY OF THE EVALUATION OF FGE.19 SUBSTANCES

Flavouring Group Evaluation 19 (FGE.19) covers 360 flavouring substances from the European Union (EU) Register that are α,β-unsaturated aldehydes or ketones, or precursors which could give rise to such carbonyl substances via hydrolysis and/or oxidation (EFSA, 2008a).

The α,β-unsaturated aldehyde and ketone structures are structural alerts for genotoxicity (EFSA, 2008a). The Panel noted that there were limited genotoxicity data on these flavouring substances but that positive genotoxicity studies were identified for some substances in the group.

The α,β-unsaturated carbonyls were subdivided into subgroups on the basis of structural similarity (EFSA, 2008a). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure–activity relationship (Q)SAR prediction of the genotoxicity of these substances was undertaken considering a number of models (DEREKfW, TOPKAT, DTU-NFI-MultiCASE Models and ISS-Local Models (Gry et al., 2007)).

The Panel noted that for most of these models internal and external validation has been performed, but considered that the outcome of these validations was not always extensive enough to appreciate the validity of the predictions of these models for these α,β-unsaturated carbonyls. Therefore, the Panel considered it inappropriate to totally rely on (Q)SAR predictions at that time and decided not to take substances through the procedure based on negative (Q)SAR predictions only.

The Panel took note of the (Q)SAR predictions obtained using two ISS Local Models (Benigni and Netzeva, 2007a, b) and four DTU–NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that data on *in vitro* and *in vivo* genotoxicity, as well as on carcinogenicity, were available for several substances. Based on these data, the Panel decided that 15 subgroups (1.1.1, 1.2.1, 1.2.2, 1.2.3, 2.1, 2.2, 2.3, 2.5, 3.2, 4.3, 4.5, 4.6, 5.1, 5.2 and 5.3) (EFSA, 2008a) could not be evaluated through the Procedure due to concern with respect to genotoxicity. Corresponding to these subgroups, 15 Flavouring Group Evaluations (FGEs) were established: FGE.200, 204, 205, 206, 207, 208, 209, 211, 215, 219, 221, 222, 223, 224 and 225.

For 11 subgroups the Panel decided, based on the available genotoxicity data and (Q)SAR predictions, that a further scrutiny of the data should take place before requesting additional data from the Flavour Industry on genotoxicity. These subgroups were evaluated in FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220. In the case of the substances in FGE.202, 214 and 218, it was concluded that a genotoxic potential could be ruled out and, accordingly, these substances would be evaluated using the Procedure. For all or some of the substances in the remaining FGEs, FGE.201, 203, 210, 212, 213, 216, 217 and 220 the genotoxic potential could not be ruled out.

To ease the data retrieval of the large number of structurally related α,β-unsaturated substances in the different subgroups for which additional data are requested, the European Food Safety Authority (EFSA) worked out a list of representative substances for each subgroup (EFSA, 2008c). Likewise an EFSA genotoxicity expert group worked out a test strategy to be followed in the data retrieval for these substances (EFSA, 2008b).

The Flavour Industry was requested to submit additional genotoxicity data according to the list of representative substances and test strategy for each subgroup.

The Flavour Industry has now submitted additional data and the present FGE concerns the evaluation of these data requested on genotoxicity.

ASSESSMENT

1. History of the evaluation of the substances in the present Flavouring Group Evaluation

In FGE.212, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) concluded that, based on available data, the concern for genotoxicity could be ruled out for *d*-carvone and five carvone derivatives in subgroup 2.6 [FL-no: 02.062, 07.147, 09.143, 09.215 and 09.870]. Therefore, these substances could be evaluated through the procedure. In the case of isophorone [FL-no: 07.126] and the structurally related substances [FL-no: 02.083, 02.101, 02.214, 07.033, 07.035, 07.094, 07.098, 07.112, 07.129, 07.140, 07.172, 07.175, 07.196, 07.202, 07.255 and 09.821] additional genotoxicity data were required according to the Test Strategy (EFSA, 2008b). In the EFSA Opinion "List of α,β-unsaturated aldehydes and ketones representative of FGE.19 substances for genotoxicity testing" (EFSA, 2008c), representative flavouring substances were selected for subgroup 2.6 (Table 1), corresponding to FGE.212, for which additional data on the genotoxicity were requested.

In FGE.212Rev1, new data on genotoxicity were submitted by Industry on the representative substance, 3,5,5-trimethylcyclohex-2-en-1-one [FL-no: 07.126]. Based on these data, the Panel concluded that the concern for genotoxicity could be ruled out for 3,5,5-trimethylcyclohex-2-en-1-one [FL-no: 07.126] and for the 10 six-carbon-ring members of subgroup 2.6 [FL-no: 02.083, 02.101, 07.035, 07.098, 07.129, 07.172, 07.175, 07.196, 07.202 and 07.255].

After revision 1 of FGE.212, one additional five-carbon ring substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], was included in the FGE.

For the two seven-carbon-ring substances, vetiverol and vetiveryl acetate [FL-no: 02.214 and 09.821] for which new genotoxicity information was submitted (IOFI, 2012) and evaluated in FGE.212Rev2, the Flavour Industry has subsequently informed EFSA that information previously submitted to the European Commission, and on which EFSA's evaluation was based, was incorrect regarding the name, structure and composition of these substances. The use of these two substances as chemically defined flavouring substances is no longer supported by the Industry (EFFA, 2014). Accordingly, these two substances will not be considered further in this FGE.

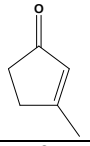
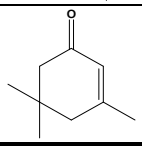
The Panel noted that the EFSA Scientific Committee adopted an opinion on carvone (EFSA Scientific Committee, 2014) and confirmed that there is no concern with respect to genotoxicity.

FGE	Adopted by EFSA	Link	No of substances
FGE.212	27 November 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902780085.htm	23
FGE.212Rev1	25 November 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1923.htm	23
FGE.212Rev2	29 January 2014	http://www.efsa.europa.eu/it/efsajournal/pub/3584.htm	24
FGE.212Rev3	5 May 2015	http://www.efsa.europa.eu/it/efsajournal/pub/4116.htm	22

Revision 3 of FGE.212 (FGE.212Rev3) is due to submission of *in vitro* genotoxicity data on the five-carbon-ring members of FGE.212. For the representative substance 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] an Ames test (Bowen, 2014) and a micronucleus assay in human peripheral blood lymphocytes (Watters, 2014) have been provided.

The new data provided for [FL-no: 07.112] are also considered to cover the evaluation of a possible genotoxicity potential of the other four five-carbon-ring members of this FGE [FL-no: 07.033, 07.094, 07.140 and 07.219].

Table 1: Representative substances for subgroup 2.6 of FGE.19 (EFSA, 2008c)

FL-no JECFA-no	Subgroup	EU Register name	Structural formula
07.112 1105	2.6	3-Methyl-2-cyclopenten-1-one	
07.126 1112	2.6	3,5,5-Trimethylcyclohex-2-en-1-one (isophorone)	

Sections 2, 3 and 4 of this opinion report the same information that was presented in FGE.212 and FGE.212Rev1. As vetiverol [FL-no: 02.214] and vetiveryl acetate [FL-no: 09.821] are no longer supported by the Industry, the text in Section 5 has been modified accordingly.

Section 6 describes the new data evaluated in FGE.212Rev3.

2. Presentation of the substances in Flavouring Group Evaluation 212Rev3

2.1. Description

The present Flavouring Group Evaluation 212 Revision 3 (FGE.212Rev3), concerns 22 substances, which are presented in Table 2. The 22 substances correspond to subgroup 2.6 of FGE.19 (EFSA, 2008a). Sixteen of these substances are α,β-unsaturated alicyclic ketones (α,β-unsaturation in the side chain) [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 07.146, 07.147, 07.172, 07.175, 07.196, 07.202, 07.219 and 07.255] and six are precursors of such ketones [FL-no: 02.062, 02.083, 02.101, 09.143, 09.215 and 09.870].

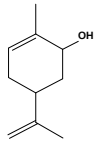
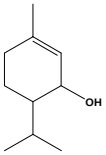
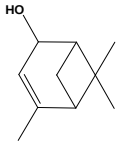
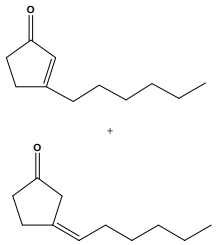
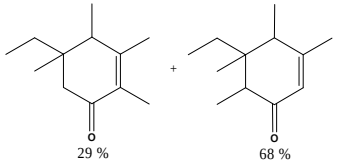
Twenty-one of the substances have previously been evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) at their 51st, 59th and 69th meetings (JECFA, 1999, 2003, 2009). A summary of their evaluation status by the JECFA is given in Table 3.

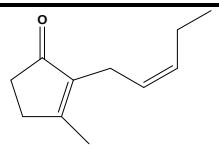
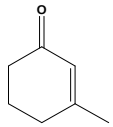
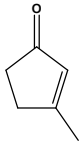
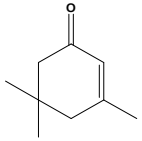
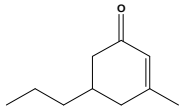
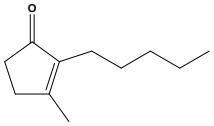
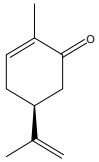
As the α,β-unsaturated ketone structure is considered to be structural alerts for genotoxicity (EFSA, 2008a), the available data on genotoxic or carcinogenic activity of the 16 ketones in FGE.212 [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 07.146, 07.147, 07.172, 07.175, 07.196, 07.202, 07.219 and 07.255] and one non-Register ketone (2,6-dimethyl-9-(1-methylethylidene)-bicyclo[5.3.0]dec-2-en-4-one), corresponding to the 22 substances in FGE.212, will be considered in this FGE.

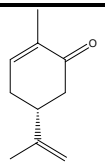
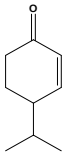
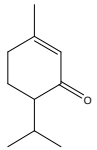
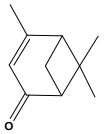
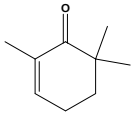
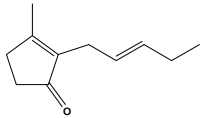
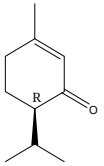
The Panel also noted that, for one substance [FL-no: 07.033], the Chemical Abstracts Service (CAS) No, name and chemical structure were not consistent (Table 2). Therefore, clarification is still needed.

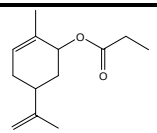
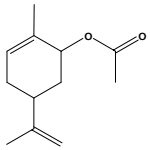
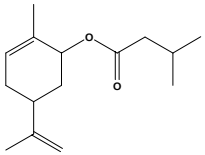
The Panel has also taken into consideration the outcome of the predictions from five selected (Q)SAR models (Benigni and Netzeva, 2007a; Gry et al., 2007; Nikolov et al., 2007) on the 15 ketones FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 07.146, 07.147, 07.172, 07.175, 07.196, 07.202 and 07.255] and one non-Register ketone (2,6-dimethyl-9-(1-methylethylidene)-bicyclo[5.3.0]dec-2-en-4-one) in this FGE. The (Q)SAR predictions for the 15 ketones and the one non-Register ketone are shown in Table 4.

Table 2: Specification summary of the substances in the Flavouring Group Evaluation 212 Revision 3

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
02.062 381	Carveol		2247 2027 99-48-9	Liquid $C_{10}H_{16}O$ 152.24	Freely soluble	226–227 IR 96%	1.493–1.497 0.947–0.953
02.083 434	*p*-Menth-1-en-3-ol		3179 10248 491-04-3	Liquid $C_{10}H_{18}O$ 154.25		232 NMR 97 %	1.4762 (25C), 0.930–0.936
02.101 1404	Pin-2-en-4-ol		3594 10304 473-67-6	Solid $C_{10}H_{16}O$ 152.24	Very slightly soluble Soluble	n.a. 63–67 NMR 95 %	n.a. n.a.
07.033 1115	Isojasmone [f]		3552 167 11050-62-7	Liquid $C_{11}H_{18}O$ 166.26		144 (13 hPa) NMR 95 %	1.472–1.477 0.917–0.924
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		3061 168 17369-60-7	Liquid $C_{12}H_{20}O$ 180.29	Slightly soluble Miscible	113–115 NMR 97 %	1.485–1.490 0.927–0.934

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
07.094 1114	3-Methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one		3196 11786 488-10-8	Liquid $C_{11}H_{16}O$ 164.25		248 NMR 98 %	1.495–1.501 0.942–0.948
07.098 1107	3-Methylcyclohex-2-en-1-one		3360 11134 1193-18-6	Liquid $C_7H_{10}O$ 110.16	Miscible Miscible	199–200 NMR 98 %	1.490–1.498 0.967–0.972
07.112 1105	3-Methyl-2-cyclopenten-1-one		3435 11137 2758-18-1	Liquid C_6H_8O 96.12		74 (20 hPa) NMR 98 %	1.485–1.491 0.968–0.975
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		3553 11918, 78-59-1	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Miscible	213–215 NMR 97 %	1.474–1.481 0.919–0.927
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		3577 3720-16-9	Liquid $C_{10}H_{16}O$ 152.23	Insoluble Miscible	242–244 NMR 95 %	1.481–1.486 0.924–0.928
07.140 1406	3-Methyl-2-pentylcyclopent-2-en-1-one		3763 1128-08-1	Liquid $C_{11}H_{18}O$ 166.26	Very slightly soluble Soluble	79 (0.2 hPa) NMR 99 %	1.676–1.682 0.911–0.917
07.146 380.1	*d*-Carvone		 2244-16-8	 $C_{10}H_{14}O$ 150.22			

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
07.147 380.2	*l*-Carvone		6485-40-1	$C_{10}H_{14}O$ 150.22			
07.172 1110	4-Isopropylcyclohex-2-en-1-one		3939 11127 500-02-7	Liquid $C_9H_{14}O$ 138.21	Insoluble Miscible	198 NMR 97 %	1.481–1.490 0.930–0.950
07.175 435	*p*-Menth-1-en-3-one		2910 2052 89-81-6	Liquid $C_{10}H_{16}O$ 152.24	Insoluble	233–235 IR 94 %	1.483–1.487 0.929–0.934
07.196 1870	Pin-2-en-4-one		4216 11186 80-57-9	Liquid $C_{10}H_{14}O$ 150.22	Insoluble Freely soluble	90 (16 hPa) NMR MS 95%	1.492–1.498 0.975–0.981
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		20013-73-4	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Freely soluble	63 (16 hPa) MS 95 %	1.470–1.476 0.924–0.930
07.219	*trans*-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		3196 11786 6261-18-3	Liquid $C_{11}H_{16}O$ 164.25	Soluble Soluble	248 MS 98 %	1.495–1.501 0.942–0.948
07.255 1856	*l*-Piperitone		4200 4573-50-6	Liquid $C_{10}H_{16}O$ 152.24	Slightly soluble Freely soluble	246 MS 99 %	1.482–1.488 0.929–0.935

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
09.143 383	Carvyl propionate		2251 424 97-45-0	Liquid $C_{13}H_{20}O_2$ 208.30	Insoluble	239 IR, 98 %	1.469–1.479 0.942–0.962
09.215 382	Carvyl acetate		2250 2063 97-42-7	Liquid $C_{12}H_{18}O_2$ 194.27	Slightly soluble	229 IR 98 %	1.473–1.479 0.964–0.970
09.870	Carvyl-3-methylbutyrate		94386-39-7	Liquid $C_{15}H_{24}O_2$ 236.37	Practically insoluble or insoluble Freely soluble	343 MS 95 %	1.462–1.468 0.932–0.938

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1 013.25 hPa, if not otherwise stated.
(d): At 20 °C, if not otherwise stated.
(e): At 25 °C, if not otherwise stated.
(f): Stereoisomeric composition not specified.
n.a.: not applicable

3. Data available to the Panel in FGE.212[9]

3.1. (Quantitative) structure–activity relationship predictions

The outcomes of the (Q)SAR predictions for possible genotoxic activity in five *in vitro* (Q)SAR models (ISS Local Model-Ames test; and DTU-NFI MultiCASE-Ames test, -Chromosomal aberration test in Chinese hamster ovary cells (CHO), -Chromosomal aberration test in Chinese hamster lung cells (CHL) and -Mouse lymphoma test) are presented in Table 4.

Positive predictions have been obtained for six substances with the MultiCASE mouse lymphoma model and for one of these substances also with the MultiCASE model on chromosomal aberrations. For the other substances, the predictions of the MultiCASE models were negative or equivocal, or the substances were out of domain. All substances were out of domain in the ISS model.

3.2. Carcinogenicity studies

Groups of 50 male and 50 female F344/N rats were administered isophorone (3,5,5-trimethylcyclohex-2-en-1-one [FL-no: 07.126]) in maize oil by gavage at dose levels of 0 (controls), 250 or 500 mg/kg body weight (bw)/day, five times per week for 103 weeks. During the study, the body weights of the male and female rats in the high-dose group were slightly lower than those of the vehicle controls. The survival of male rats in the high-dose group was significantly lower than that of the vehicle-treated controls after week 96. Dosed male rats showed a variety of proliferative lesions of the kidney (tubular cell hyperplasia, 0/50, 1/50, 4/50 in the control, 250 mg/kg and 500 mg/kg groups, respectively; tubular cell adenoma, 0/50, 0/50, 2/50; tubular cell adenocarcinoma, 0/50, 3/50, 0/50; epithelial hyperplasia of the renal pelvis, 0/50, 5/50, 5/50). Dosed male rats also exhibited increased mineralisation of the medullary collecting ducts (1/50, 31/50, 20/50) and male rats in the low-dose group showed a more severe nephropathy than is commonly seen in ageing F344/N rats. The incidence of carcinomas of the preputial gland was significantly increased ($P < 0.03$) in male rats in the high-dose group (0/50, 0/50, 5/50 in the control, 250 mg/kg and 500 mg/kg groups, respectively). With the exception of a moderate increase in nephropathy (21/50, 39/50, 32/50 in the control, 250 mg/kg and 500 mg/kg groups, respectively), female rats did not show chemically related increased incidences of neoplastic or non-neoplastic lesions (NTP, 1986).

Groups of 50 male and 50 female $B6C3F_1$ mice were administered isophorone (3,5,5-trimethylcyclohex-2-en-1-one [FL-no: 07.126]) in maize oil by gavage at dose levels of 0 (controls), 250 or 500 mg/kg bw/day, five times per week for 103 weeks. During the study, the body weights of female mice in the high-dose group were slightly lower than those of the vehicle controls. The survival of male mice was low, whereas there was a significant trend towards increased survival of dosed female mice relative to that of the vehicle controls. In male mice of the high-dose group, isophorone exposure was associated with an increased incidence of hepatocellular adenomas and carcinomas (18/48, 18/50, 29/50 in the control, 250 mg/kg and 500 mg/kg groups, respectively) and of mesenchymal tumours of the integumentary system (fibroma, fibrosarcoma, neurofibrosarcoma or sarcoma: 6/48, 7/50, 14/50). An increased incidence of lymphomas or leukaemias was noted in male mice in the low-dose group (8/48, 18/50, 5/50 in the control, 250 mg/kg and 500 mg/kg groups, respectively). Coagulative necrosis (3/48, 10/50, 11/50) and hepatocytomegaly (23/48, 39/50, 37/50) were observed more frequently in the livers of dosed male mice than in vehicle controls. No compound-related neoplastic or non-neoplastic lesions associated with isophorone exposure were seen in female mice (NTP, 1986).

The Panel concluded that isophorone increased the incidences of renal tubular cell adenomas and adenocarcinomas and of carcinomas of the preputial gland in male rats but not in female rats. In male

[9] The data presented in Section 3 are cited from the first version of the present FGE.212. These data are the basis of the conclusions in FGE.212 requesting additional genotoxicity data.

mice, but not in females, it produced increased incidences of hepatocellular adenomas and carcinomas, mesenchymal tumours in the integumentary system and malignant lymphomas.

The Panel agrees with the authors of the National Toxicology Program (NTP) report, who concluded that "under the conditions of these 2-year gavage studies, there was some evidence of carcinogenicity of isophorone in male F344/N rats as shown by the occurrence of renal tubular cell adenomas and adenocarcinomas in animals given 250 or 500 mg/kg bw per day; carcinomas of the preputial gland were also observed at increased incidence in male rats given 500 mg/kg bw. There was no evidence of carcinogenicity in female F344/N rats given 250 or 500 mg/kg bw per day. For male $B6C3F_1$ mice, there was equivocal evidence of carcinogenicity of isophorone as shown by an increased incidence of hepatocellular adenomas or carcinomas (combined) and of mesenchymal tumours in the integumentary system in animals given 500 mg/kg bw per day and by an increase in malignant lymphomas in animals given 250 mg/kg bw per day. There was no evidence of carcinogenicity of isophorone in female $B6C3F_1$ mice given 250 or 500 mg/kg bw per day."

Groups of 50 male and 50 female $B6C3F_1$ mice (7 weeks old) were administered 0, 375 or 750 mg/kg bw *d*-carvone [FL-no: 07.146] in maize oil by gavage, five days per week for 103 weeks. The mean body weights of dosed and control male and female mice were similar throughout most of the study. The survival of females in both the low-dose and the high-dose groups was significantly greater than that of the controls. No differences in survival were observed between any groups of male mice. Atrophy of the olfactory epithelium and hyperplasia of the underlying Bowman's glands occurred together, with high incidence, in both sexes and in both dosed groups. This effect was found because of a local effect of *d*-carvone caused by reflux of the gavage material when the gavage needle was withdrawn. No increases in tumour incidences were seen in mice administered *d*-carvone. The incidences of primary neoplasms in male mice and the total numbers of primary neoplasms were significantly lower in the dosed groups than in the vehicle controls (NTP, 1990).

The Panel concluded that *d*-carvone was not carcinogenic in mice under the study conditions. It agrees with the authors of the NTP report, who concluded that "under the conditions of these 2-year gavage studies, there was no evidence of carcinogenic activity of *d*-carvone for male or female $B6C3F_1$ mice administered 375 or 750 mg/kg, 5 days per week for 2 years."

Study validation and results are presented in Table 5.

3.3. Genotoxicity studies

Studies are available for four substances in subgroup 2.6. For tetramethyl ethylcyclohexenone (mixture of isomers) [FL-no: 07.035], one *in vitro* and one *in vivo* study have been evaluated.

Seven *in vitro* and three *in vivo* studies are available for 3,5,5-trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone).

Three *in vitro* studies are available concerning *d*-carvone [FL-no: 07.146] and two *in vitro* studies concerning *l*-carvone [FL-no: 07.147].

Study validation and results are presented in Tables 6 and 7.

3,5,5-Trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone) did not induce gene mutations in bacteria, but it did induce mutations in mammalian cells in a mouse lymphoma thymidine kinase (MLTK) assay in the absence of metabolic activation (it was not tested in the presence of metabolic activation) (NTP, 1986). No mutations in the MLTK assay were observed in a study carried out by O'Donoghue et al. (1988) at comparable concentrations. Isophorone induced chromosomal aberrations (CA) in CHL fibroblasts with and without metabolic activation (Matsuoka et al., 1996) and sister chromatid exchanges (SCE) in CHO cells without metabolic activation (Gulati et al., 1989). CA were not observed in two other studies (NTP, 1986; Gulati et al., 1989); however, the validity of the results was limited because the types of aberrations were not reported. Isophorone did not induce unscheduled

DNA synthesis (UDS) in rat hepatocytes *in vitro*. *In vivo*, isophorone was tested negative in a sex-linked recessive lethal mutation assay in *Drosophila* (Foureman et al., 1994) and in two micronucleus assays in mice (McKee et al., 1987; O'Donoghue et al., 1988). However, the *Drosophila* assay has only limited relevance and the micronucleus assays were of limited validity.

Negative results were also observed with tetramethyl ethylcyclohexenone [FL-no: 07.035] in bacteria, in a sex-linked recessive lethal mutation assay in *Drosophila* (Wild et al., 1983) and in a mouse micronucleus assay (Wild et al., 1983); however, a mixture of isomers was tested and the studies were of only limited validity.

d-Carvone [FL-no: 07.146] was not mutagenic in bacteria but induced SCE and CA in CHO cells in the presence and absence of metabolic activation, respectively (NTP, 1990).

3.4. Conclusion on genotoxicity and carcinogenicity

The Panel concluded that 3,5,5-trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone) is genotoxic *in vitro*, but a final conclusion on its genotoxicity *in vivo* could not be drawn based on the data available. It is carcinogenic in male rats and male mice. It was also predicted to be genotoxic in one of the four MultiCASE models (whereas it was out of domain in the ISS model).

d-Carvone [FL-no: 07.146] is genotoxic *in vitro* but no *in vivo* data were available. *d*-Carvone is not carcinogenic in mice and was predicted to be non-genotoxic in the four MultiCASE models (whereas it was out of domain in the ISS model). No data are available on *l*-carvone. However, *in vivo* studies in humans show that the metabolism of ingestion-correlated amounts of *d*- or *l*-carvone occurs via a major oxidative pathway of the isopropylene side chain, yielding diol and two carboxylic acids, irrespective of the stereochemical difference between the two parent isomers of carvone (Engel, 2001). Accordingly, the results for *d*-carvone can be used for *l*-carvone as well.

The negative results reported from *in vivo* studies on the genotoxicity of tetramethyl ethylcyclohexenone [FL-no: 07.035] are of only limited validity.

3.5. Conclusion

The present Flavouring Group Evaluation 212 (FGE.212) concerns 23 substances. The 23 substances correspond to subgroup 2.6 of FGE.19. Fifteen of these substances are α,β-unsaturated alicyclic ketones [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 07.146, 07.147, 07.172, 07.175, 07.196, 07.202 and 07.255] and eight are precursors for such ketones [FL-no: 02.062, 02.083, 02.101, 02.214, 09.143, 09.215, 09.821 and 09.870].

d-Carvone [FL-no: 07.146] was found to be genotoxic *in vitro*. However, *d*-carvone is not carcinogenic in mice. Therefore, the Panel concluded that this substance, together with the structurally related *l*-carvone, as well as carveol and the carvyl derivatives [FL-no: 02.062, 07.147, 09.143, 09.215 and 09.870], could be evaluated through the Procedure.

Isophorone [FL-no: 07.126] (3,5,5-trimethylcyclohex-2-en-1-one) is genotoxic *in vitro* and, since there is some evidence of carcinogenicity in male rats and equivocal evidence of carcinogenicity in male mice, and since a non-threshold mechanism could not be excluded based on the data currently available, the Panel concluded that additional data are required for isophorone in order to clarify whether or not genotoxicity occurs *in vivo* and if there is a threshold for the effects observed in the target organs in the long-term bioassays. Therefore, an *in vivo* Comet assay in F344/N rats covering these target organs is required in addition to an *in vivo* bone marrow assay with oral application.

Owing to structural similarities and lack of data, the remaining substances cannot presently be evaluated through the Procedure [FL-no: 02.083, 02.101, 02.214, 07.033, 07.035, 07.094, 07.098, 07.112, 07.129, 07.140, 07.172, 07.175, 07.196, 07.202, 07.255 and 09.821]. Additional data on genotoxicity are requested for representative substances of this subgroup according to the opinion of

the Panel on the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19 (EFSA, 2008b).

4. Additional data considered by the Panel in FGE.212Rev1[10]

4.1. Presentation of the additional data

Honma et al. (1999a, b) found that isophorone did not clearly induce mutations in the mouse lymphoma assay (MLA) following 3 hour treatments, but observed that it was mutagenic after 24 hour treatments in the absence of S9. Although only graphs are plotted, it seems that increases in mutation frequency (MF) that exceeded the Global Evaluation Factor (GEF) occurred at around 1250–1500 μg/ml at which point toxicity (as measured by relative survival) reached 70–90 %.

The NTP conducted a mouse bone marrow chromosomal aberration (CA) study on isophorone. Groups of 8 male B6C3F1 mice (larger group sizes than required by OECD) were dosed via intraperitoneal (i.p.) injection with isophorone at 125, 250 and 500 mg/kg bw. The standard protocol for *in vivo* CA is not given on the NTP website. However, based on Shelby and Witt (1995), animals should have been sampled at 17 hours and, if negative, also at 36 hours. The data on the NTP website are for bone marrow sampled at 36 hours only. It is therefore possible that a 17-hour sample was also taken, and found to be negative, and that the data were not posted. Fifty cells per animal were scored for CA and no increases in CA were seen. No measures of toxicity were recorded, but i.p. dosing should have guaranteed systemic exposure. The control CA frequency was normal (2.75 %) and the positive control (dimethylbenzanthracene) produced a significant response in CA frequency.

A DNA binding study was conducted in which F344 rats and B6C3F1 mice (the strains used in the NTP carcinogenicity study) were exposed to isophorone (Thier et al., 1990). Animals of both sexes were dosed once or five times by gavage with 500 mg/kg bw of unlabelled isophorone spiked with [1,3,5-^{14}C]-isophorone (specific activity 52 mCi per mmol, 1.92 GBq per mmol). An additional group of acute dosed male rats received undiluted ^{14}C-isophorone for increased sensitivity. Rats and mice were maintained for 24 hours in closed metabolic cages. Twenty-four hours after exposure, livers and kidneys (the tumour target tissues) were removed from the animals. DNA was isolated through hydroxyapatite chromatography and radioactivity was measured by liquid scintillation counting. No positive controls were included. In addition, no untreated controls were included, but, except for the liver sample of one mouse in the five times dose group, radioactivity values were within 2σ of background (6 dpm). Radioactivity values therefore did not indicate significant attachment of radioactivity to DNA. From these results it can be concluded that neither isophorone nor its metabolites bind covalently to DNA.

In addition, a report by Morishita et al. (1997) submitted to EPA (1997) is relevant and appears to have been previously submitted only as an abstract. This study was designed to investigate whether isophorone and/or α2μ-globulin[11] might be involved in the induction of preputial gland tumours in F344 rats (10/sex/dose group). A series of experiments was performed in order to study several parameters including:

- Binding of isophorone to DNA in the kidney and preputial gland. Groups of 10 male rats were dosed by gavage with 500 mg/kg of [^{14}C]-isophorone (specific activity 14.65 mCi/mmol; 100 μCi/animal). Positive control animals were dosed with ^{3}H-labelled methyl nitrosourea.
- DNA adduct detection by ^{32}P-postlabelling in young adult male and female rats (7 per group) dosed by gavage with 0, 250 or 500 mg/kg isophorone for five days.

[10] The data presented in Section 4 are cited from revision 1 of FGE.212 (FGE.212Rev1). These data are the basis for the conclusions in FGE.212Rev1 requesting additional genotoxicity data.

[11] Since interaction with α2μ-globulin is not of direct relevance for the evaluation of genotoxic potential, this information is omitted from this study summary.

Extraction of preputial gland and kidney DNA from rats treated with single 500 mg/kg labelled doses yielded no evidence of isophorone binding to DNA, whereas the positive control showed significant binding to DNA of preputial gland and kidney. These negative results with isophorone were confirmed in the ^{32}P-postlabelling assays.

In addition Industry has also asked whether the information submitted for isophorone (cyclohexenyl derivative) could also be applied to evaluate the genotoxic potential of the five-carbon-membered ring substances (i.e. cyclopentenyl derivatives) in subgroup 2.6 (letter from the European Flavour and Fragrance Association (EFFA) to EFSA, dated 14 April 2010). This request was supported by the argumentation that there is structural resemblance with respect to steric hindrance around the α,β-unsaturated double bond. In addition, Industry argued that the π-conjugation systems in these molecules are very nearly planar and, therefore, that the reactivity and genotoxic potentials of the five- and six-membered ring systems would be similar. No further data were provided to substantiate this argumentation.

4.2. Discussion of the additional data

Conflicting results were reported in two valid studies with the mouse lymphoma assay (MLA): one negative (O'Donoghue et al., 1988) and one positive (NTP, 1986), at comparable concentrations. Mixed results were also reported in two studies of limited validity: one negative (Honma et al., 1999a) and one positive (Honma et al., 1999b). Another negative result was reported in a study (McKee et al., 1987) the validity of which cannot be evaluated. In the light of the clearly negative results in two valid bacterial gene mutation tests (Ames test) and in a valid sex-linked recessive lethal mutations test (SLRL) in *Drosophila*, and taking into account the lack of specificity and high sensitivity of the MLA, overall the results currently available are considered of questionable relevance. The Panel agrees that isophorone demonstrates some genotoxic activity *in vitro* but that the new data demonstrate lack of clastogenicity *in vivo*. In addition, the new DNA-binding data from two separate studies provide convincing evidence that isophorone does not induce tumours via a genotoxic mechanism. On the basis of these data it may be argued that there is no need to perform further *in vivo* genotoxicity studies, such as the Comet assay or bone marrow micronucleus test. Thus, based on the data available, the Panel concluded that there is no concern with respect to genotoxicity of isophorone.

4.3. Conclusion on additional data

Since, based on the additional information, the concern for the genotoxic potential for isophorone has been alleviated, a genotoxic potential can also be ruled out for the other six-carbon members of subgroup 2.6 related to isophorone [FL-no: 02.083, 02.101, 07.035, 07.098, 07.126, 07.129, 07.172, 07.175, 07.196, 07.202 and 07.255].

The Panel also concluded that isophorone can be considered only representative of the six-carbon ring members of subgroup 2.6. The argumentation of Industry that this conclusion should be extended to the cyclopentenyl derivatives in this subgroup [FL-no: 07.033, 07.094, 07.112 and 07.140] was considered too limited, given the lack of supporting experimental data. Therefore, additional genotoxicity tests are still required for the representative substance [FL-no: 07.112] already chosen by the Panel. Alternatively, a more thorough explanation (physico-chemical parameters; experimental underpinning) of the proposed similar reactivity of six- and five-membered ring substances should be provided by Industry. In addition, for the substance [FL-no: 02.214] additional data on genotoxicity are still required.

5. Additional data considered by the Panel in FGE.212Rev2

In response to the EFSA request in FGE.212 and FGE.212Rev1 for additional genotoxicity data for subgroup 2.6, the Flavour Industry (IOFI, 2012) has submitted *in vitro* genotoxicity data on vetiveryl acetate [FL-no: 09.821], which is structurally related to vetiverol [FL-no: 02.214]. These data were evaluated in FGE.212Rev2, but, after the publication of the opinion, the Flavour Industry informed EFSA that information previously submitted to the European Commission, and on which EFSA's

previous evaluation was based, was incorrect regarding the name, structure and composition of these substances. The use of these two substances as chemically defined flavouring substances is no longer supported by Industry (EFFA, 2014). Accordingly, these two substances will not be considered further in this FGE.

6. Additional data considered by the Panel in FGE.212Rev3

6.1. Bacterial reverse mutation assay

In order to investigate the potential of 3-methyl-2-cyclopenten-1-one [FL-no: 17.112] to induce gene mutations in bacteria, an Ames test was performed in accordance with OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP) in five strains of *Salmonella Typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) in the presence or absence of metabolic activation by S9-mix in two separate experiments. In experiment 1, the "plate incorporation assay" was applied. In experiment 2, treatment with S9-mix included a pre-incubation step (20 minutes at 37 °C). Seven different concentrations of the test substance were tested using appropriate positive control chemicals and purified water as a negative control. The highest concentration selected was 5000 µg/plate (range 5 to 5000 µg/plate and 80 to 5000 µg/plate in experiments 1 and 2, respectively). All positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix, while the negative controls were within the normal ranges. After treatment with 3-methyl-2-cyclopenten-1-one in experiment 1, evidence of toxicity, in the form of diminished background bacterial lawn, was observed at 5000 µg/plate in both the presence and absence of S9-mix in all strains; in addition, strain TA98 also showed toxicity at 160, 500 and 1600 µg/plate. At most experimental points, at the same concentrations the number of revertant colonies was relatively low. In experiment 2, toxicity was observed at 5000 µg/plate in all strains but TA102, in both the presence and absence of S9-mix (Bowen, 2014).

No increase in revertant colony numbers was observed at any concentration tested in either the presence and absence of S9-mix. Therefore, it was concluded that 3-methyl-2-cyclopenten-1-one has no mutagenic activity under the conditions employed.

6.2. *In vitro* micronucleus assay in human lymphocytes

The *in vitro* micronucleus assay was carried out in accordance with OECD Test Guideline 487 (OECD, 2010) and following GLP. Duplicate cultures of human peripheral blood lymphocytes, prepared from the pooled blood of two female donors and stimulated with phytohaemagglutinin (PHA), were treated with purified water (negative control), 3-methyl-2-cyclopenten-1-one or appropriate positive controls (mitomycin C and noscapine as clastogenic and aneugenic chemicals, respectively, in the absence of S9-mix; cyclophosphamide as a clastogenic chemical in the presence of S9-mix). A single experiment was performed 48 hours after mitogen stimulation, following two treatment schedules: 3 + 21 hours in the presence and absence of S9-mix and 24 + 0 hours without S9-mix. Micronuclei were analysed at three concentrations (600, 800 and 962 µg/ml; the highest concentration is equivalent to 10 mM) chosen on the basis of a preliminary cytotoxicity range-finder experiment. Applying the 3 + 21 hours treatment, the cultures were exposed to 3-methyl-2-cyclopenten-1-one for 3 hours in either the presence or the absence of the S9-mix. In the 24 + 0 hours treatment cultures were continuously exposed to 3-methyl-2-cyclopenten-1-one for 24 hours without the S9-mix. In all cases, the cells were harvested 24 hours after the beginning of treatment (i.e. 72 hours after culture initiation). Four thousand binucleated cells per concentration were analysed. All positive control chemicals induced statistically significant increases in the frequency of micronucleated cells, confirming the sensitivity of the tests and the efficacy of the S9-mix, while the negative controls were within 95th percentile of the current observed historical vehicle control ranges. At any concentration tested in both the presence and absence of S9-mix, the frequency of binucleated cells with micronuclei was comparable to that of negative controls (values of $p \leq 0.05$ were considered significant). It was concluded that 3-methyl-2-cyclopenten-1-one did not induce micronuclei in cultured human peripheral blood lymphocytes when tested at concentrations up to 10 mM under the experimental conditions employed (Watters, 2014).

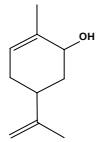
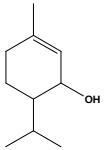
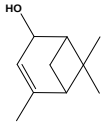
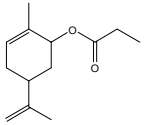
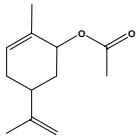
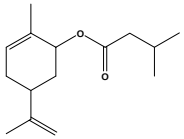
The results of the two studies described above are summarised in Table 8.

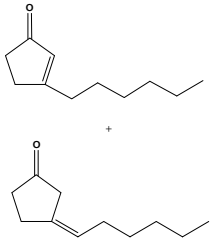
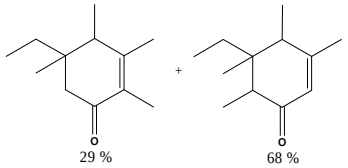
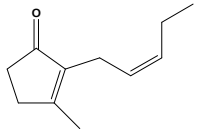
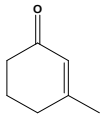
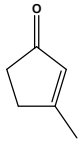
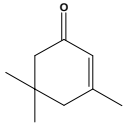
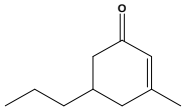
CONCLUSION

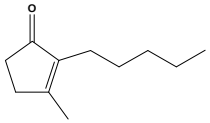
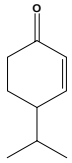
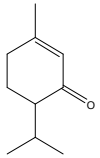
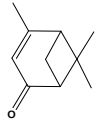
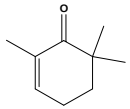
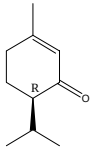
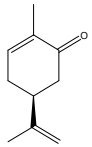
The genotoxicity of the flavouring substance 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] was assessed by means of two *in vitro* assays (gene mutations in bacteria and micronuclei in human lymphocytes). 3-Methyl-2-cyclopenten-1-one did not induce gene mutations in bacteria with or without metabolic activation when tested under the conditions employed in the study as presented by the applicant. Nor did it induce micronuclei in cultured human blood lymphocytes under the test conditions employed with or without metabolic activation for this study. Therefore, there is no concern with respect to genotoxicity and the substance 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] can be evaluated through the Procedure. This conclusion is also valid for the other four five-carbon ring substances isojasmone [FL-no: 07.033], 3-methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one [FL-no: 07.094], 3-methyl-2-pentylcyclopent-2-en-1-one [FL-no: 07.140] and trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219]. Based on the available data all 22 substances of this FGE are no longer of concern with respect to genotoxic and, therefore, they can be evaluated through the Procedure.

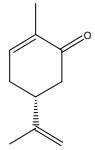
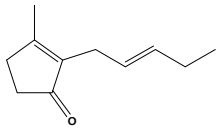
SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (JECFA, 1999, 2003, 2009)

Table 3: Summary of safety evaluation applying the Procedure

FL-no JECFA-no	EU Register name	Structural formula	MSDI [a] (μg/capita/day)	Class [b], Evaluation procedure path [c]	Outcome on the named compound [d] or [e]
02.062 381	Carveol		9.5 140	Class I A3: intake below threshold	d
02.083 434	*p*-Menth-1-en-3-ol		0.012 0.02	Class I A3: intake below threshold	d
02.101 1404	Pin-2-en-4-ol		0.012 0.2	Class I A3: intake below threshold	d
09.143 383	Carvyl propionate		0 0.04	Class I A3: intake below threshold	d
09.215 382	Carvyl acetate		4 36	Class I A3: intake below threshold	d
09.870	Carvyl-3-methylbutyrate		0.0012	Class I A3: intake below threshold	d

FL-no JECFA-no	EU Register name	Structural formula	MSDI [a] (μg/capita/day)	Class [b], Evaluation procedure path [c]	Outcome on the named compound [d] or [e]
07.033 1115	Isojasmone		0.37 0.01	Class II A3: Intake below threshold	d
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		7.8 0.2	Class II A3: Intake below threshold	d
07.094 1114	3-Methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one		13 7.2	Class II A3: intake below threshold	d
07.098 1107	3-Methylcyclohex-2-en-1-one		0.012 0.1	Class II A3: intake below threshold	d
07.112 1105	3-Methyl-2-cyclopenten-1-one		0.06 ND	Class II A3: intake below threshold	d
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		4.6 0.1	Class II A3: intake below threshold	d
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		0.097 4.1	Class II A3: intake below threshold	d

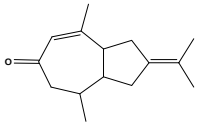
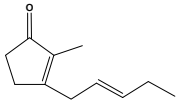
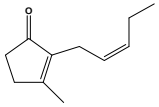
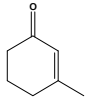
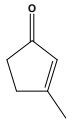
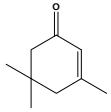
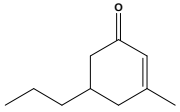
FL-no JECFA-no	EU Register name	Structural formula	MSDI [a] (µg/capita/day)	Class [b], Evaluation procedure path [c]	Outcome on the named compound [d] or [e]
07.140 1406	3-Methyl-2-pentylcyclopent-2-en-1-one		0.34 0.2	Class II A3: intake below threshold	d
07.172 1110	4-Isopropylcyclohex-2-en-1-one		0.0012 0.001	Class II A3: intake below threshold	d
07.175 435	*p*-Menth-1-en-3-one		44 10	Class II A3: intake below threshold	d
07.196 1870	Pin-2-en-4-one		15	Class II A3: intake below threshold	d
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		0.12	Class II A3: intake below threshold	d
07.255 1856	*l*-Piperitone		12	Class II A3: intake below threshold	d
07.146 380.1	*d*-Carvone		2390 9900	Class II A3: intake above threshold, A4: not endogenous, A5: adequate NOAEL exists	d

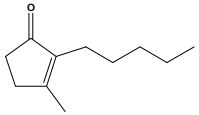
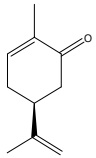
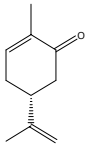
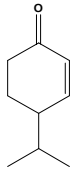
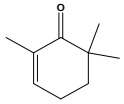
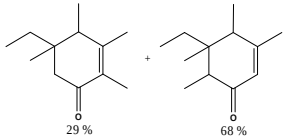
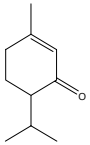
FL-no JECFA-no	EU Register name	Structural formula	MSDI [(a)] (µg/capita/day)	Class [(b)], Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]
07.147 380.2	*l*-Carvone		2390 9900	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d
07.219	*trans*-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		4.7	No evaluation	

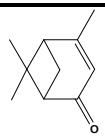
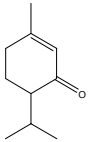
(a): EU MSDI: amount added to food as flavour in (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita/day.
(b): Thresholds of concern: Class I = 1 800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.

QSAR PREDICTIONS ON MUTAGENICITY IN FIVE MODELS FOR 16 KETONES FROM SUBGROUP 2.6

Table 4: QSAR predictions on mutagenicity in five models for subgroup 2.6

FL-no JECFA-no	EU Register name	Structural formula [a]	ISS Local Model Ames Test TA100 [b]	MultiCASE Ames test [c]	MultiCASE Mouse lymphoma test [d]	MultiCASE Chromosomal aberration test in CHO [e]	MultiCASE Chromosomal aberration test in CHL [f]
Not in Register	2,6-Dimethyl-9-(1-methylethylidene)-bicyclo[5.3.0]dec-2-en-4-one		OD	NEG	NEG	NEG	NEG
07.033 1115	Isojasmone		OD	NEG	NEG	NEG	NEG
07.094 1114	3-Methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one		OD	NEG	OD	NEG	NEG
07.098 1107	3-Methylcyclohex-2-en-1-one		OD	NEG	POS	NEG	EQU
07.112 1105	3-Methyl-2-cyclopenten-1-one		OD	NEG	POS	NEG	EQU
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		OD	NEG	POS	NEG	EQU
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		OD	NEG	POS	NEG	EQU

FL-no JECFA-no	EU Register name	Structural formula [a]	ISS Local Model Ames Test TA100 [b]	MultiCASE Ames test [c]	MultiCASE Mouse lymphoma test [d]	MultiCASE Chromosomal aberration test in CHO [e]	MultiCASE Chromosomal aberration test in CHL [f]
07.140 1406	3-Methyl-2-pentylcyclopent-2-en-1-one		OD	NEG	OD	NEG	NEG
07.146 380.1	*d*-Carvone		OD	NEG	NEG	NEG	NEG
07.147 380.2	*l*-Carvone		OD	NEG	NEG	NEG	NEG
07.172 1110	4-Isopropylcyclohex-2-en-1-one		OD	NEG	NEG	NEG	EQU
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		OD	NEG	OD	NEG	NEG
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		OD	NEG	NEG	NEG	NEG
07.255	*l*-Piperitone		OD	NEG	OD	NEG	EQU

FL-no JECFA-no	EU Register name	Structural formula [a]	ISS Local Model Ames Test TA100 [b]	MultiCASE Ames test [c]	MultiCASE Mouse lymphoma test [d]	MultiCASE Chromosomal aberration test in CHO [e]	MultiCASE Chromosomal aberration test in CHL [f]
07.196	Pin-2-en-4-one		OD	NEG	POS	NEG	POS
07.175	*p*-Menth-1-en-3-one		OD	NEG	POS	NEG	OD

(a): Structural group 2.6: α,β-unsaturated alicyclic ketones.
(b): Local model on aldehydes and ketones, Ames test TA100 (NEG, negative; POS, positive; OD, out of domain).
(c): MultiCASE Ames test (OD, out of domain; POS, positive; NEG, negative; EQU, equivocal).
(d): MultiCASE mouse lymphoma test (OD, out of domain; POS, positive; NEG, negative; EQU, equivocal).
(e): MultiCASE chromosomal aberration in CHO (OD, out of domain; POS, positive; NEG, negative; EQU, equivocal).
(f): MultiCASE chromosomal aberration in CHL (OD, out of domain; POS, positive; NEG, negative; EQU, equivocal).
OD: out of applicability domain: not matching the range of conditions where a reliable prediction can be obtained in this model. These conditions may be physico-chemical, structural, biological, etc.

CARCINOGENICITY STUDIES CONSIDERED BY THE PANEL IN FGE.212

Table 5: Carcinogenicity studies

Chemical name [FL-no]	Species, sex, no/group	Route	Dose levels	Duration	Results	Reference	Comments [(a)]
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Rats, male + female, 50/sex/group	Gavage in maize oil	0 (controls), 250 or 500 mg/kg bw/day, five times per week	103 weeks	Males: increased incidences of renal tubular cell adenomas and adenocarcinomas and of carcinomas of the preputial gland Females: no carcinogenic effect	NTP (1986)	Valid
	Mice, male + female, 50/sex/group	Gavage in maize oil	0 (controls), 250 or 500 mg/kg bw/day, five times per week	103 weeks	Males: Increased incidences of hepatocellular adenomas and carcinomas, mesenchymal tumours in the integumentary system and malignant lymphomas Females: no carcinogenic effect	NTP (1986)	Valid
d-Carvone [07.146]	Mice, male + female, 50/sex/group	Gavage	0, 375 or 750 mg/kg bw/day, five times per week	103 weeks	Males and females: no increases in tumour incidences	NTP (1990)	Valid

(a): Validity of genotoxicity studies:

- Valid.
- Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and/or there is limited documentation).
- Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or the test system is inappropriate).
- Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too few experimental details provided).

GENOTOXICITY DATA (*IN VITRO*) CONSIDERED BY THE PANEL IN FGE.212

Table 6: Summary of genotoxicity data (*in vitro*)

Chemical name [FL-no]	Test system	Test object	Concentration	Reported result	Reference	Comments [(e)]
Tetramethyl ethylcyclohexenone (mixture of isomers) [07.035]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Five concentrations up to cytotoxicity or maximum 3 600 µg/plate	Negative [(a)]	Wild et al. (1983)	Limited validity (no TA102 or *Escherichia coli*); possibly slightly low maximal concentration tested
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	33–10 000 µg/plate	Negative [(a)]	Mortelmans et al. (1986)	Valid
	Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–10 000 µg/plate	Negative [(a)]	NTP (1986)	NTP study carried out in accordance with standard US EPA Guideline; result is considered valid
	Mutation	L5178YTk+/– mouse lymphoma cells	67–810 µg/ml	Negative [(b)]	McKee et al. (1987)	Validity cannot be evaluated (tested with S9; abstract only with very limited information)
	Mutation	L5178YTk+/– mouse lymphoma cells	130–1 300 µg/ml	Negative [(c)]	McKee et al. (1987)	Validity cannot be evaluated (tested without S9; abstract only with very limited information)
	Mutation	L5178YTk+/– mouse lymphoma cells	0.089–0.89 µl/ml	Negative [(c)]	O'Donoghue et al. (1988)	Valid according to current guidelines
	Mutation	L5178YTk+/– mouse lymphoma cells	0.13–1.3 µl/ml	Negative [(b)]	O'Donoghue et al. (1988)	Valid according to current guidelines
	Mutation	L5178YTk+/– mouse lymphoma cells	1 200 µg/ml	Positive [(b)]	NTP (1986)	NTP study carried out in accordance with standard US EPA Guideline; not tested with S9. Result is considered valid

Chemical name [FL-no]	Test system	Test object	Concentration	Reported result	Reference	Comments [e]
	Mutation	L5178YTk+/– mouse lymphoma cells	Not reported (however, up to cytotoxic concentrations) for three-hour exposure	Negative [a]	Honma et al. (1999a)	Limited validity since data were presented in a summarised table format only (as a result of an international collaborative study)
	Mutation	L5178YTk+/– mouse lymphoma cells	Up to 1 500 µg/ml	Positive [b]	Honma et al. (1999b)	Limited validity. Isophorone was mutagenic after 24-hour treatments in the absence of S9. Although only graphs are plotted, it seems that increases in MF that exceeded the GEF occurred at around 1 250–1 500 µg/ml, at which point toxicity (measured by relative survival) reached 70–90 %
	Chromosomal aberration	Chinese hamster ovary cells	5–1 600 µg/ml	Negative [a]	Gulati et al. (1989)	Limited validity (not clear if gaps were included in the scores)
	Chromosomal aberration	Chinese hamster ovary cells	250–1 600 µg/ml	Negative [a]	NTP (1986)	NTP study carried out in accordance with standard US EPA Guideline; result is considered valid
	Chromosomal aberration	Chinese hamster lung fibroblasts	0–1 250 [b] µg/ml 0–1 500 [c] µg/ml	Positive [a]	Matsuoka et al. (1996)	Valid
	Chromosomal aberration	Chinese hamster lung fibroblasts	250–1 000 mg/ml	Negative [a]	Matsuoka et al. (1996)	Valid. Exposed to isophorone without metabolic activation for 24 or 48 hours; cytotoxic at highest concentrations
	Sister chromatid exchange	Chinese hamster ovary cells	5–1 600 mg/ml	Positive [b] [d]	Gulati et al. (1989)	Valid (positive, –S9; negative, +S9)

Chemical name [FL-no]	Test system	Test object	Concentration	Reported result	Reference	Comments [(e)]
	Sister chromatid exchange	Chinese hamster ovary cells	160–1 000 mg/ml	Negative [(a)]	NTP (1986)	NTP study carried out according to standard US EPA Guideline; result is considered valid
	Unscheduled DNA synthesis	Rat hepatocytes	0.005–0.4 µl/ml	Negative	O'Donoghue et al. (1988)	Valid according to current guidelines
	Unscheduled DNA synthesis	Rat hepatocytes	5–200 µl/ml	Negative [(a)]	McKee et al. (1987)	Validity cannot be evaluated (abstract only with very limited information)
Carvone (isomer not specified)	Gene mutation	*S. typhimurium* TA1535, TA1537, TA98, TA100	3 µmol/plate	Negative	Florin et al. (1980)	Insufficient validity (spot test, not in accordance OECD Guideline, methods and results insufficiently reported). Isomer (D or L) not reported
	Rec assay	*Bacillus subtilis* H17 (rec+) and M45 (rec–)	0.6 ml/disc	Negative	Matsui et al. (1989)	The test system used is considered inappropriate
d-Carvone [07.0146]	Gene mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	333 µg/plate	Negative [(a)]	NTP (1990)	Valid
	Gene mutation (pre-incubation)	*S. typhimurium* TA1535, TA98, TA100, TA1537	560 µg/plate	Negative	Mortelmans et al. (1986)	Valid
	Sister chromatid exchange	Chinese hamster ovary cells	502 µg/ml	Positive [(a)]	NTP (1990)	Valid
	Chromosomal aberration	Chinese hamster ovary cells	400 µg/ml	Positive [(a)]	NTP (1990)	Valid

(a): With and without metabolic activation.
(b): Without metabolic activation.
(c): With metabolic activation.
(d): Cytotoxic at next highest dose tested (1600 mg/ml).
(e): Validity of genotoxicity studies:

- Valid.
- Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and/or there is limited documentation).
- Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or the test system is inappropriate).
- Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too few experimental details provided).

GENOTOXICITY DATA (*IN VIVO*) CONSIDERED BY THE PANEL IN FGE.212 AND IN FGE.212REV1

Table 7: Summary of genotoxicity data (*in vivo*)

Chemical name [FL-no]	Test system	Test object	Route	Dose	Result	Reference	Comments [(a)]
Tetramethyl ethylcyclohexenone (mixture of isomers) [07.035]	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	Feed	10 mM	Negative	Wild et al. (1983)	Limited validity (low number of chromosomes, limited reporting)
	Micronucleus formation	Mouse bone marrow	i.p.	180, 307 and 450 mg/kg bw	Negative	Wild et al. (1983)	Limited validity. Only analysis at one time point; no PCE/NCE ratio reported
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Sex-linked recessive lethal mutation	*Drosophila melanogaster*		2000 and 1250 ppm	Negative	Foureman et al. (1994)	Valid; however, only limited relevance
	Micronucleus formation	CD-1 mice	i.p.	540 mg/kg bw (MTD)	Negative	McKee et al. (1987)	Validity cannot be evaluated. Abstract only; very limited information, no data on PCE/NCE ratio
		CD-1 mice	i.p.	0.54 ml/kg bw	Negative	O'Donoghue et al. (1988)	Limited validity. Only one dose level tested, this dose level corresponded to the LD20; sample schedule inadequate
	Chromosomal aberration	B6C3F1 mice	i.p.	125, 250 and 500 mg/kg bw	Negative	NTP website	Valid. Submitted by the Flavour Industry in 2009. The standard protocol for *in vivo* CA is not given on the NTP website. However, based on Shelby and Witt (1995), animals should have been sampled at 17 hours and, if negative, also at 36 hours. The data on the NTP website are only for bone marrow sampled at 36 hours. It is therefore possible that a 17-hour sample was also taken, and found to be negative, but that these data were not posted. Fifty cells per animal

Chemical name [FL-no]	Test system	Test object	Route	Dose	Result	Reference	Comments [(a)]
							were scored for CA and no increases in CA were seen. No measures of toxicity were recorded, but i.p. dosing should have guaranteed systemic exposure
	DNA binding	F344 rats	Gavage	500 mg unlabelled isophorone/kg bw spiked with ^{14}C-isophorone (0.4 mCi/rat)	Negative	Thier et al. (1990)	Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed
	DNA binding	B6C3F1 mice	Gavage	500 mg unlabelled isophorone/kg bw spiked with ^{14}C-isophorone (0.08 mCi/mouse)	Negative	Thier et al. (1990)	Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed
	DNA binding	F344 rats (10 males)	Gavage	500 mg/kg bw ^{14}C-isophorone (0.1 mCi/rat)	Negative	Morishita et al. (1997); cited by EPA (1997)	Valid. Preputial glands and kidneys were analysed
	DNA adducts (^{32}P-postlabelling)	F344 rats (7 males and 7 females per dose group)	Gavage	0 and 500 mg/kg/day for 5 days.	Negative	Morishita et al. (1997); cited by EPA (1997)	Valid. Preputial glands were analysed

(a): Validity of genotoxicity studies:

- Valid.
- Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and/or there is limited documentation).
- Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or the test system is inappropriate).
- Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too few experimental details provided).

GENOTOXICITY DATA (*IN VITRO*) CONSIDERED BY THE PANEL IN FGE.212REV3

Table 8: Summary of genotoxicity data (*in vitro*)

Chemical name [FL-no]	Test system	Test object	Concentration	Reported result	Reference	Comments
3-Methyl-2-cyclopenten-2-one [07.112]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA102	5–5 000 µg/plate	Negative [(a)]	Bowen (2014)	TA98 showed toxicity at 160 µg/ml
	Micronucleus assay	Human peripheral blood lymphocytes	600, 800 and 962 µg/plate	Negative	Watters (2014)	

(a): With and without metabolic activation.

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1. Benigni R and Netzeva T, 2007a. Report on a QSAR model for prediction of genotoxicity of α,β-unsaturated aldehydes in *S. Typhimurium* TA100 and its application for predictions on α,β-unsaturated aldehydes in Flavouring Group Evaluation 19 (FGE.19). Unpublished report submitted by FLAVIS Secretariat to EFSA.

2. Benigni R and Netzeva T, 2007b. Report on a QSAR model for prediction of genotoxicity of α,β-unsaturated ketones in *S. Typhimurium* TA100 and its application for predictions on α,β-unsaturated aldehydes in Flavouring Group Evaluation 19 (FGE.19). Unpublished report submitted by FLAVIS Secretariat to EFSA.

3. Bowen R, 2014. 3-methyl-2-cyclopenten-1-one: Bacterial reverse mutation assay. Covance Laboratories Ltd. Study no 8302484. 23 October 2014. Unpublished report submitted by EFFA to DG SANTE.

4. EFFA (European Flavour Association), 2010. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

5. EFFA (European Flavour Association), 2012a. Private Communication forwarded to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark. Dated 17 January 2012, 14, 23 and 24 February 2012 and 19 March 2012. Specification data related to substances in FGE.29.Rev1: [FL-no: 01.015]; FGE.09Rev4 [FL-no: 07.059, 09.843 and 09.920] and FGE.51Rev1 [FL-no: 07.034, 07.035, 07.095, 07.129, 07.172, 07.257 and 09.930]. FLAVIS/8.143.

6. EFFA (European Flavour Association), 2012b. E-mail from EFFA to EFSA/CEF Secretariat, dated 15 March 2012. Information on isomerism of five substances evaluated in FGE.47 [FL-no: 02.119, 07.171, 07.196, 09.888 and 09.848]. FLAVIS/8.149.

7. EFFA (European Flavour Association), 2014. Letter from EFFA to EFSA on re-evaluation of flavouring substances FGE.19 Subgroup 2.6a: Vetiverol [FL-no: 02.214] and Vetiveryl acetate [FL-no: 09.821]. Dated 19 June 2014.

8. Gocke E, 2000. Evaluation of vetiveryl acetate + 1 % tocopherol alpha for mutagenic activity in the Ames test. F. Hoffmann La-Roche Ltd. Study no 373M00. 16 October 2000. Unpublished report submitted by EFFA to FLAVIS Secretariat.

9. IOFI (International Organization of the Flavor Industry), 2012. Flavouring Group Evaluation 19 Subgroup 2.6 Part A—Flavouring Substances of the Chemical Group 3 (Annex I of 1565/2000/EC) Alicyclic α,β-unsaturated aldehydes, ketones and related substances with the α,β-conjugation in the ring or in the side chain. 17/12-2012. FLAVIS/8.183.

10. Morishita K, Schoonhoven R and Swenberg JA, 1997. Mechanistic studies on isophorone and preputial gland carcinomas. Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, N.C. 25 March 1997. Unpublished report submitted by EFFA to FLAVIS secretariat.

11. Morris A and Durward R, 2010. Chromosome aberration test in CHO cell: *in vitro*. Vetiveryl acetate. Harlan Laboratories Ltd. Project no 2153/0007. 16 April 2010. Unpublished report submitted by EFFA to FLAVIS Secretariat.

12. Morris A, 2011. Chromosome aberration test in human lymphocytes *in vitro*. Vetiveryl acetate. Harlan Laboratories Ltd. Project no 41101037. 22 November 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

13. Poth A, 2003. *Salmonella Typhimurium* reverse mutation assay with vetiveryl acetate stab. (18 months). R C C Cytotest Cell Research GMBH. Study no 846892. 14 March 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat.

14. Scheerbaum D, 2001. Reverse Mutation Assay (Ames test) with *Salmonella Typhimurium*. Vetiverylacetate extra. Dr. U. Noack-Laboratorium für Angewandte Biologie. Project no 001006HR. 5 March 2001. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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ABBREVIATIONS

bw	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHL	Chinese hamster lung cells
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	deoxyribonucleic acid
DTU	Danish Technical University
EC	European Commission
EFFA	European Flavour and Fragrance Association
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GEF	global evaluation factor
ID	identity
IOFI	International Organization of the Flavour Industry
i.p.	intraperitoneal
IR	infrared spectroscopy
ISS	Istituto Superiore di Sanità
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MLA	mouse lymphoma assay
MLTK	mouse lymphoma thymidine kinase
MS	mass spectrometry
MTD	maximum tolerated dose
NCE	normochromatic erythrocytes
NFI	National Food Institute
NMR	nuclear magnetic resonance
No	number
NOAEL	No Observed Adverse Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	polychromatic erythrocytes

(Q)SAR	quantitative structure–activity relationship
SCE	sister chromatid exchange
WHO	World Health Organization